ELISA kit User Manual

Porcine Cortisol ELISA Kit

Catalog No.: 22921

96T

For Research Use Only

Not for In Vitro Diagnostic Use

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Intended use: The ELISA Kit is to be used for quantitative determination of Porcine Cortisol in serum, Plasma and other biological fluids. Kit is intended for research use only not for diagnostics.

1. Principles of Method

The Porcine Cortisol Elisa Kit is an Vitro enzyme-linked immunosorbent assay for the quantitative measurement of Porcine Cortisol in serum, plasma, tissue lysates, cell culture supernatants and urine. The Porcine Cortisol polyclonal antibodies are pre-coated onto 96-well plate. Standard and samples are aspirated into the wells and Porcine Cortisol present in a sample is bound to the wells by the immobilized antibody. The biotinylated detection antibodies are added to the wells and then followed by washing with PBS or TBS buffer. After washing away unbound biotinylated antibody, Avidin-Biotin-Peroxidase Complex is added to the wells. The wells are washed again, a TMB substrate solution is added to the wells and the color changes after adding acidic stop solution. The intensity of the color is proportional to the amount of Porcine Cortisol bound in samples and measured at 450nm±10nm.

2. Reagents and Materials Provided

Composition	Quantity	Composition	Quantity
Pre-coated Micro titer plate, 96 wells	1	Sample Diluent Buffer	2 X 15ml
Standards	2	ABC Diluent Buffer	1 X 12ml
Detection Antibody	1 X 120 ul	Antibody Diluent Buffer	1 X 12ml
Avidin-Biotin-Peroxidase Complex(ABC)1:100	1 X 120 ul	Stop solution	1 X 10ml
TMB color developing reagent A	1 X 10ml	TBS Diluent/Wash Buffer(25X)	1 X 20ml
TMB color developing reagent B	1 X 1.5 ml	Instruction manual	1

Note: Standard：Frozen dried, TMB color developing reagent A：Avoid light

3. Materials Required But Not Provided

① Micro titer plate reader in standard size.

② Polypropylene tubes for diluting and for aliquoting standard.

③ Distilled or de-ionized water.

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④ Calibrated, adjustable precision pipettes, preferably with disposable plastic tips.

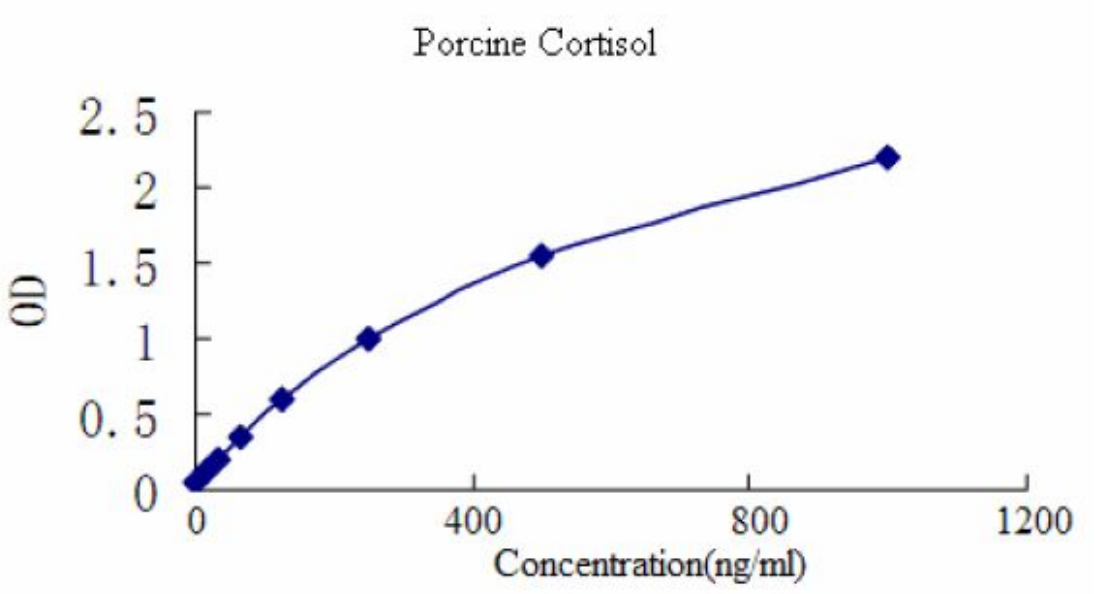
4. Performance Characteristic

Normal Range: 1000ng/ml-15.6ng/ml

Sensitivity: <5ng/ml

Specificity: The ELISA Kit shows no cross reactivity with any of the cytokines.

5. Reference curve



6. Storage and stability

All the components in the kit can be stored up to 1 year at -20℃ and 4 weeks at 2-8℃. Please avoid repeated freeze-thaw cycles and do not mix reagents from different kits unless they have the same lot numbers.

7. Reagents Preparation

① Plate washing

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Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.35 ml PBS or TBS buffer for 1~2 minutes, then discard the rinse solution. Repeat this process for several times.

② **Sample Preparation and Storage**

Stored samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

Cell culture supernatant, tissue lysates or body fluids: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C.

Serum: Allow the serum to clot in a serum separator tube (about 2 hours or 4℃) at room temperature. Centrifuge at approximately 1000 X g for 10 min. Analyze the serum immediately or aliquot and store frozen at -20°C.

Plasma: Collect plasma using heparin, EDTA, citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 min of collection. Analyze immediately or aliquot and store frozen at -20°C.

Sample Dilution Guideline

User needs to estimate the concentration of the target protein in the samples and select proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve.

③ **Reagent Preparation and Storage**

A. Preparation of the standard: Standard solution should be prepared no more than 2 hours prior to the experiment. Add 1 ml sample diluent buffer into one tube, dissolve the standard thoroughly and make times dilution.

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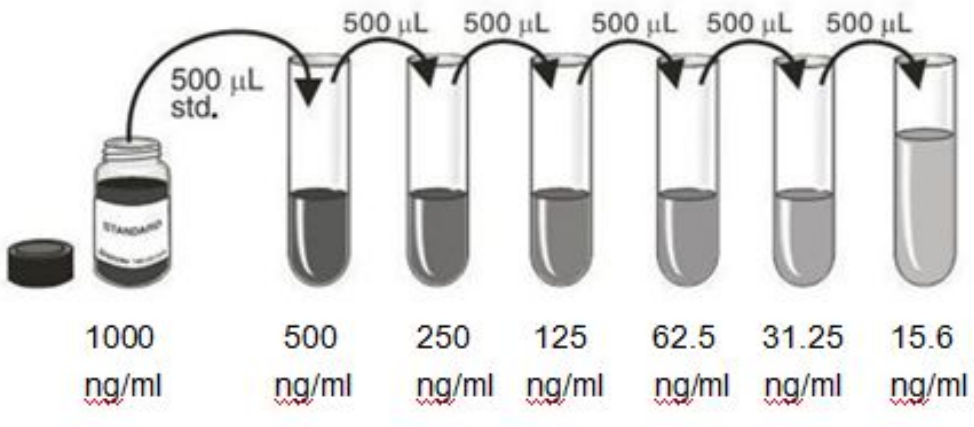

B. Preparation of biotinylated anti-Porcine Cortisol antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment. Note: please make centrifuging for the vial before preparing.

a. The total volume should be (100ul) or 0.1ml/well x (the number of wells). (Allowing 0.1-0.2ml more than total volume)

b. Biotinylated anti-Porcine Cortisol antibody should be diluted in 1:100(1+99) with the antibody diluent buffer and mixed thoroughly.

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment. Note: please make centrifuging for the vial before preparing.

a. The total volume should be: (100µl) or 0.1ml/well x (the number of wells). (Allowing 0.1-0.2ml more than total volume)

b. Avidin-Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 (1+99) with the ABC dilution buffer and mixed thoroughly.

D. Preparation of TMB working solution: transfer 9 volumes of TMB color developing **Reagent A** in one volume of TMB color developing **Reagent B** for 30 minutes in 37℃ before using to make TMB substrate, mixing thoroughly.

8. Assay Procedure

The user should decide sample dilution fold by crude estimation of Porcine Cortisol amount in samples.

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① Aliquot 100μl per well of the grades Porcine Cortisol standard solutions into the pre-coated 96-well plate. Add 100ul of the sample diluent buffer into the control wells. Add 100μl of each properly diluted sample of sera, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well.

② Seal the plate with the cover and incubate at 37°C for 90 min.

③ Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time, washing twice with 300μl of wash buffer.

④ Add 100μl of biotinylated anti-Porcine Cortisol antibody working solution into each well and incubate the plate at 37°C for 60 min.

⑤ Wash the plate three times with 0.01M TBS and each time let washing buffer stay in the wells for 1 min.

⑥ Add 100μl of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.

⑦ Wash plate 5 times with 0.01M TBS and each time let washing buffer stay in the wells for 1-2 min.

⑧ Add 90μl of prepared TMB color developing agent into each well and incubate plate at 37°C for 10-15 minutes away from light, observe the color at all times, when shades of blue can be seen in the wells with the three-four most concentrated Porcine Cortisol standard solutions; the other wells show no obvious color.

⑨ Add 100μl of prepared stop solution into each well to stop the reaction. The color changes into yellow immediately.

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⑩ Read the O.D. absorbance at 450nm in a micro plate reader within 30 min after adding the stop solution or even 620-630nm Secondary wavelength.

⑪ The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Porcine Cortisol concentration of the samples can be interpolated from the standard curve.

Summary

Prepare reagents, samples and standards.

⇩

Add 100μl prepared samples or standards and incubate the plate at 37℃ for 90min, wash plate twice with 300 μl TBS.

⇩

Add 100μl biotinylated antibodies and incubate the plate at 37℃ for 60min, wash plate 3 times with TBS.

⇩

Add 100μl ABC working solution and incubate the plate at 37℃ for 30 min, wash plate five times with TBS.

⇩

Add 90μl TMB color developing agent and incubate at 37℃.

⇩

Add 100μl stop solution.

⇩

Read the O.D. absorbance at 450nm within 30 min.

⇩

Calculate the Porcine Cortisol concentration in the samples by plotting graph between Concentrations and corresponding absorbencies of Standards.

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